Biomedical Science

Thrombopoietin and Platelet Development

KENNETH KAUSHANSKY, MD, *Seattle, Washington*

Blood cell production, termed hematopoiesis, is a complex process dependent on bone marrow stromal cells, polypeptide growth factors, and primitive stem and progenitor cells.[1,2] Pioneering work in the early and mid-1960s suggested that marrow contains undifferentiated cells that develop into the mature blood elements.[3-5] Over the ensuing 30 years, a number of soluble and membrane-bound polypeptides have been characterized that support the survival, proliferation, and differentiation of primitive marrow cells into erythrocytes, neutrophils, eosinophils, basophils, monocytes or macrophages, and lymphocytes. Nearly all of these molecules have been cloned.

In more recent years, the availability of these recombinant cytokines and purified hematopoietic progenitors has led to the concept that stem cells are supported by two types of growth factors, one that acts on several types of primitive progenitor cells and one that acts specifically on a single lineage late in its developmental pathway. For example, erythrocyte development is thought to be supported by the early action of stem cell factor (also called steel factor, kit ligand, and mast cell growth factor)[6] and erythropoietin, which acts to induce the terminal phases of erythrocyte development.[7] For neutrophils, interleukin (IL) 3 or granulocyte-macrophage colony-stimulating factor (CSF) and granulocyte (G)-CSF appear to fill these early and late supportive roles.[8-10] Many of these cytokines, especially the late-acting hormones erythropoietin and G-CSF, have found important clinical roles in the stimulation of erythrocyte and leukocyte production in states of natural and iatrogenic marrow failure.[11,12] Unfortunately, the cytokines responsible for megakaryocyte and platelet development have been more difficult to identify.

In the late 1960s and early 1970s, several investigators showed that the plasma of thrombocytopenic animals contained a substance that induced an increased uptake of selenium 75- or sulfur 35-labeled methionine into the platelets of normal recipients, a process thought to represent new platelet production. This substance was called "thrombopoietin" and appeared to support the late stages of megakaryocyte maturation.[13,14] The marrow of recipient animals was characterized by increases in megakaryocyte size and polyploidy, rather than by changes in cell number. Despite this early progress, the subsequent steps of biochemical purification and cloning of thrombopoietin proved elusive. Using in vivo assays or surrogate in vitro methods, several investigators in the mid-1980s were able to partially purify a thrombopoietic material from thrombocytopenic plasma.[15-18] Such preparations could recapitulate in vivo findings; the partially purified material induced megakaryocyte ploidy and cytoplasmic maturation in suspension cultures of rodent megakaryocytes. Unfortunately, none of these studies led to a purification of thrombopoietin sufficient to provide the amino acid sequence.

By adapting many of the techniques useful for myeloid and erythroid cell culture, several investigators established that megakaryocyte production could be supported in serum or plasma containing cultures by IL-3[19] and to a lesser extent by stem cell factor.[20] Both of these cytokines appeared to act early in the developmental process; cell numbers were increased, but in the absence of plasma, megakaryocytes failed to mature. These results suggested that IL-3 or stem cell factor (or both) was the early acting factor(s) for megakaryocyte development, but that plasma contained a distinct substance responsible for megakaryocyte maturation.[21] These conclusions were consistent with results from studies of plasma thrombopoietin. Despite this greater understanding of the characteristics of megakaryocyte and platelet development, investigators were no further along in cloning a distinct thrombopoietin. Many in the field began to think that if modern technology could not clone it, it did not exist.

In the late 1980s and early 1990s, several cytokines were identified that displayed pleotropic biologic activities including effects on megakaryocyte production. Interleukins 6 and 11 and leukocyte inhibitory factor all were shown to augment megakaryocyte formation in response to IL-3 or stem cell factor.[22-24] Moreover, these cytokines appeared to affect megakaryocyte maturation, causing increases in cell size and ploidy both in vitro and in vivo. Although it was clear that neither IL-6 nor IL-11 was the thrombopoietic substance identified in plasma,[25,26] many in the field began to think that together these pleotropic cytokines might play the role of a thrombopoietin, further reinforcing the growing suspicion that a distinct lineage-specific megakaryocyte maturation factor did not exist. If so, however, megakaryocyte and

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From the Division of Hematology, University of Washington School of Medicine, Seattle.

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Reprint requests to Kenneth Kaushansky, MD, Division of Hematology, Box 357710, University of Washington School of Medicine, Seattle, WA 98195.

ABBREVIATIONS USED IN TEXT

CSF = colony-stimulating factor
G-CSF = granulocyte colony-stimulating factor
IL = interleukin

platelet development was organized in a far different way from erythrocyte or leukocyte production.

Occasionally in science, findings from one field, although interesting and important in their own right, have a profound catalytic effect on a seemingly unrelated area of research. Work with the murine retrovirus MPLV had such an effect on megakaryocytopoiesis. In the late 1980s, a retroviral complex was described that induced an acute myeloproliferative syndrome in recipient mice.[27] In 1990 the transforming gene responsible for the disease was identified,[28] and the corresponding human proto-oncogene, c-*mpl*, cloned from human erythroleukemia cells in 1992.[29] On the basis of spatially conserved cysteine residues and a pentapeptide juxtamembrane sequence, features characteristic of a large number of growth factor receptors,[30] it was suggested that c-*mpl* encoded a cytokine receptor. The ligand for the polypeptide was not known, however; c-*mpl* encoded an orphan receptor.

Based on its cell of origin, a biphenotypic leukemia that can be induced to display megakaryocytic features,[31] we and others hypothesized that c-*mpl* might encode the thrombopoietin receptor. On this basis, many groups sought to clone its ligand. Additional data emerged suggesting that this hypothesis was correct. First, c-*mpl* expression appeared to be limited to normal megakaryocytes, their precursors, or their progeny.[29,32] When a group of leukemic cell lines was surveyed for c-*mpl* expression, only those that displayed characteristics of the megakaryocytic lineage were found positive.[32] Next, genetic engineering of a hybrid receptor composed of the IL-4–receptor extracellular domain and the intracellular domain of c-*mpl* functioned to transmit a proliferative signal in a leukemic cell line.[33] This result confirmed that c-*mpl* encoded a growth-promoting receptor. But the strongest evidence that c-*mpl* and its ligand functioned in megakaryocyte development came from studies in which c-*mpl* expression was interrupted. By using antisense deoxyoligonucleotides, investigators showed that megakaryocytic, but not erythroid or myeloid, development of highly selected marrow progenitor and stem cells was markedly reduced if c-*mpl* expression was inhibited.[34] With these data as further validation of the hypothesis, work progressed at a frantic pace. Within two years of the cloning of c-*mpl*, five separate groups, using at least three distinct strategies, reported the sequence of the mpl ligand.[35-39] The recombinant protein displayed all of the activities proposed for thrombopoietin.

Using the murine protein, we were able to show that the mpl ligand increased megakaryocyte size, ploidy, and surface membrane expression of platelet-specific glycoproteins.[40] Moreover, when administered to normal mice, the protein substantially increased the number of marrow and splenic megakaryocytic progenitors, megakaryocytes, and peripheral blood platelets.[35,40] Qualitatively similar results have been reported using the human homologue.[36,37,39] Other workers demonstrated that levels of the plasma mpl ligand were inversely related to platelet mass,[41] a finding later confirmed and extended by others.[42,43] Although thrombopoietin was not originally thought to stimulate the proliferation of megakaryocytic progenitor cells, the mpl ligand was found to be a potent stimulus of megakaryocyte colony formation. More recently, the elimination of c-*mpl* expression in genetically engineered mice and studies using a soluble, neutralizing form of the receptor have shown that thrombopoietin is essential for normal megakaryocyte and platelet development.[44,45] Together these findings clearly establish that the mpl ligand is identical to plasma thrombopoietin. To paraphrase Descartes, "It has been cloned; therefore, it exists."

As expected from this discussion of its history, thrombopoietin appears to play an important physiologic role in platelet homeostasis. With the use of a biologic assay, it has been shown that during the course of bone marrow transplantation, thrombopoietin levels are inversely related to the peripheral blood platelet count.[42] Moreover, in a number of other thrombocytopenic disorders (including idiopathic thrombocytopenic purpura), plasma thrombopoietin levels are elevated. The precise inverse relationship between plasma levels of thrombopoietin and the platelet count has led some investigators to propose that platelets regulate the levels of the hormone responsible for their production.[43] Because these data are based on biologic assays of thrombopoietin activity in which platelet-derived inhibitors of thrombopoietic activity could mask the true levels of the hormone, additional studies are required to validate this model. If correct, however, it would appear that thrombopoietin is regulated by some of the same mechanisms proposed for the regulation of macrophage CSF, the cytokine responsible for monocyte and macrophage development.[46]

Thrombopoietin is expressed in many tissues. Northern blot analysis has demonstrated specific messenger RNA in liver, kidney, muscle, and several other tissues.[35,36,47] This widespread distribution seems to be due to its expression in fibroblasts and endothelial cells (unpublished observations),[47] components of the stroma of all organs. Whether its expression is regulated in one or many of these organs, however, is currently under intense study. Congenital deficiency of the hormone may exist; amegakaryocytic thrombocytopenia is a candidate for such a disorder. Whether an acquired state of Tpo deficiency exists, akin to erythropoietin deficiency in renal failure, awaits further study.

Like the clinical successes of erythropoietin and G-CSF, maturation factors for erythrocytes and neutrophils, respectively, thrombopoietin is envisioned to play an important clinical role in states of natural and iatrogenic marrow failure. Toward this end, preclinical trials of the agent have been under way for the past year. Using a combined chemotherapy and radiation protocol that induces profound but nonlethal pancytopenia in mice, we have shown that the daily administration of thrombopoietin

speeds the recovery of platelet counts by nearly two weeks.[48] In a lethal model of pancytopenia, it has been shown that thrombopoietin reduces mortality primarily through a favorable effect on platelet recovery.[49] Studies have been extended to dogs and nonhuman primates. For example, it has been shown that thrombopoietin nearly eliminates radiation-induced thrombocytopenia in baboons.[50] None of these studies have reported any acute or chronic toxic effects. From these studies, it is likely that thrombopoietin will effectively reduce the thrombocytopenic complications of aggressive chemotherapy. Based on these favorable results, clinical trials of the agent are now beginning in patients with cancer. We may be on the verge of a new era of aggressive therapy for cancer.

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REFERENCES

1. Metcalf D: Hematopoietic regulators: Redundancy or subtlety? Blood 1993; 82:3515-3523

2. Ogawa M: Differentiation and proliferation of hematopoietic stem cells. Blood 1993; 81:2844-2853

3. Till JE, McCulloch EA: A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. Radiat Res 1961; 14:213-222

4. Bradley TR, Metcalf D: The growth of mouse bone marrow cells in vitro. Aust J Exp Biol Med Sci 1966; 44:287-299

5. Pluznik DH, Sachs L: The cloning of normal 'mast' cells in tissue culture. J Cell Physiol 1966; 66:319-328

6. Dai CH, Krantz SB, Zsebo KM: Human burst-forming units-erythroid need direct interaction with stem cell factor for further development. Blood 1991; 78:2493-2497

7. Geissler K, Stockenhuber F, Hinterberger W, Balske B, Lechner K: Recombinant human erythropoietin: A multipotential hemopoietic growth factor in vivo and in vitro, *In* Schaefer RM, Heidland A, Horl WH (Eds): Erythropoietin in the Nineties—Contributions to Nephrology, Vol 87. Basel, Karger, 1990, pp 1-10

8. Bot FJ, van Eijk L, Schipper P, Löwenberg B: Effects of human interleukin-3 on granulocytic colony-forming cells in human bone marrow. Blood 1989; 73:1157-1160

9. Metcalf D: Clonal analysis of proliferation and differentiation of paired daughter cells: Action of granulocyte-macrophage colony-stimulating factor on granulocyte-macrophage precursors. Proc Natl Acad Sci USA 1980; 77: 5327-5330

10. Metcalf D, Nicola NA: Proliferative effects of purified granulocyte colony-stimulating factor (G-CSF) on normal mouse hemopoietic cells. J Cell Physiol 1983; 116:198-206

11. Dessypris EN, Krantz SB: Erythropoietin: Regulation of erythropoiesis and clinical use. Adv Pharmacol 1990; 21:127-147

12. Lieschke GJ, Burgess AW: Granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor. N Engl J Med 1992; 327: 99-106

13. McDonald TP: Thrombopoietin: Its biology, purification, and characterization. Exp Hematol 1988; 16:201-205

14. Hill RJ, Levin J: Regulators of thrombopoiesis: Their biochemistry and physiology. Blood Cells 1989; 15:141-166

15. McDonald TP, Clift R, Cottrell M: A four-step procedure for the purification of thrombopoietin. Exp Hematol 1989; 17:865-871

16. Hill RJ, Leven RM, Levin FC, Levin J: The effect of partially purified thrombopoietin on guinea pig megakaryocyte ploidy in vitro. Exp Hematol 1989; 17:903-907

17. Tayrien G, Rosenberg RD: Purification and properties of a megakaryocyte stimulatory factor present both in the serum-free conditioned medium of human embryonic kidney cells and in thrombocytopenic plasma. J Biol Chem 1987; 262:3262-3268

18. Erickson-Miller CL, Ji H, Parchment RE, Murphy MJ Jr: Megakaryocyte colony-stimulating factor (Meg-CSF) is a unique cytokine specific for the megakaryocyte lineage. Br J Haematol 1993; 84:197-203

19. Kavnoudias H, Jackson H, Ettlinger K, Bertoncello I, McNiece I, Williams N: Interleukin 3 directly stimulates both megakaryocyte progenitor cells and immature megakaryocytes. Exp Hematol 1992; 20:43-46

20. Avraham H, Vannier E, Cowley S, et al: Effects of the stem cell factor, c-*kit* ligand, on human megakaryocytic cells. Blood 1992; 79:365-371

21. Williams N, Jackson H, Walker F, Oon SH: Multiple levels of regulation of megakaryocytopoiesis. Blood Cells 1989; 15:123-133

22. Ikebuchi K, Wong GG, Clark SC, Ihle JN, Hirai Y, Ogawa M: Interleukin 6 enhancement of interleukin 3-dependent proliferation of multipotential hemopoietic progenitors. Proc Natl Acad Sci USA 1987; 84:9035-9039

23. Paul SR, Bennett F, Calvetti JA, et al: Molecular cloning of a cDNA encoding interleukin 11, a stromal cell-derived lymphopoietic and hematopoietic cytokine. Proc Natl Acad Sci USA 1990; 87:7512-7516

24. Metcalf D, Waring P, Nicola NA: Actions of leukaemia inhibitory factor on megakaryocyte and platelet formation. Ciba Found Symp 1992; 167:174-187

25. Williams N: Is thrombopoietin interleukin 6? Exp Hematol 1991; 19: 714-718

26. Straneva JE, van Besien KW, Derigs G, Hoffman R: Is interleukin 6 the physiological regulator of thrombopoiesis? Exp Hematol 1992; 20:47-50

27. Wendling F, Varlet P, Charon M, Tambourin P: A retrovirus complex inducing an acute myeloproliferative leukemia disorder in mice. Virology 1986; 149:242-246

28. Souyri M, Vigon I, Penciolelli JF, Tambourin P, Wendling F: A putative truncated cytokine receptor gene transduced by the myeloproliferative leukemia virus immortalizes hematopoietic progenitors. Cell 1990; 63:1137-1147

29. Vigon I, Mornon JP, Cocault L, et al: Molecular cloning and characterization of *MPL*, the human homolog of the v-*mpl* oncogene: Identification of a member of the hematopoietic growth factor receptor superfamily. Proc Natl Acad Sci USA 1992; 89:5640-5644

30. Cosman D: The hematopoietin receptor superfamily. Cytokine 1993; 5: 95-106

31. Long MW, Heffner CH, Williams JL, Peters C, Prochownik EV: Regulation of megakaryocyte phenotype in human erythroleukemia cells. J Clin Invest 1990; 85:1072-1084

32. Vigon I, Dreyfus F, Melle J, et al: Expression of the c-*mpl* proto-oncogene in human hematologic malignancies. Blood 1993; 82:877-883

33. Skoda RC, Seldin DC, Chiang MK, Peichel CL, Vogt TF, Leder P: Murine c-*mpl*: A member of the hematopoietic growth factor receptor superfamily that transduces a proliferative signal. EMBO J 1993; 12:2645-2653

34. Methia N, Louache F, Vainchenker W, Wendling F: Oligodeoxynucleotides antisense to the proto-oncogene c-*mpl* specifically inhibit in vitro megakaryocytopoiesis. Blood 1993; 82:1395-1401

35. Lok S, Kaushansky K, Holly RD, et al: Cloning and expression of murine thrombopoietin cDNA and stimulation of platelet production in vivo. Nature 1994; 369:565-568

36. de Sauvage FJ, Hass PE, Spencer SD, et al: Stimulation of megakaryocytopoiesis and thrombopoiesis by the c-Mpl ligand. Nature 1994; 369:533-538

37. Bartley TD, Bogenberger J, Hunt P, et al: Identification and cloning of a megakaryocyte growth and development factor that is a ligand for the cytokine receptor Mpl. Cell 1994; 77:1117-1124

38. Sohma Y, Akahori H, Seki N, et al: Molecular cloning and chromosomal localization of the human thrombopoietin gene. FEBS Lett 1994; 353:57

39. Kuter DJ, Beeler DL, Rosenberg RD: The purification of megapoietin: A physiological regulator of megakaryocyte growth and platelet production. Proc Natl Acad Sci USA 1994; 91:11104-11108

40. Kaushansky K, Lok S, Holly RD, et al: Promotion of megakaryocyte progenitor expansion and differentiation by the c-*mpl* ligand thrombopoietin. Nature 1994; 369:568-571

41. Wendling F, Maraskovsky E, Debili N, et al: c-Mpl ligand is a humoral regulator of megakaryocytopoiesis. Nature 1994; 369:571-574

42. Nichol J, Hokom MM, Hornkohl A, et al: Megakaryocyte growth and development factor. J Clin Invest 1995; 95:2973-2978

43. Kuter DJ, Rosenberg RD: The reciprocal relationship of thrombopoietin (c-Mpl ligand) to changes in the platelet mass during busulfan-induced thrombocytopenia in the rabbit. Blood 1995; 85:2720-2730

44. Gurney AL, Carver-Moore K, de Sauvage FJ, Moore MW: Thrombocytopenia in c-*mpl*-deficient mice. Science 1994; 265:1445-1448

45. Kaushansky K, Broudy VC, Lin N, et al: Thrombopoietin, the Mpl-ligand, is essential for full megakaryocyte development. Proc Natl Acad Sci USA 1995; 92:3234-3238

46. Bartocci A, Mastrogiannis DS, Migliorati G, Stockert RJ, Wolkoff AW, Stanley ER: Macrophages specifically regulate the concentration of their own growth factor in the circulation. Proc Natl Acad Sci USA 1987; 84:6179-6183

47. McCarty JM, Kaushansky K: Murine Thrombopoietin mRNA Levels Are Modulated by Platelet Count (Abstr). Read at the American Society of Hematology meeting, Nashville, Tennessee, December 1994

48. Sprugel KH, Humes JM, Grossman A, Ren HP, Kaushansky K: Recombinant Thrombopoietin Stimulates Rapid Platelet Recovery in Thrombocytopenic Mice (Abstr). Blood 1994; 84(suppl 1):242A

49. Ulich TR, del Castillo J, Yin S, et al: Megakaryocyte growth and development factor ameliorates carboplatin-induced thrombocytopenia in mice. Blood 1995; 86:971-976

50. Farese AM, Hunt P, Grab LB, Matlick DR, MacVittie TJ: Modulation of Megakaryocytopoiesis in Normal and Sublethally Irradiated Nonhuman Primates By Recombinant Human Megakaryocyte Growth and Development Factor (r-HuMGDF). Presented at the American Society for Blood and Marrow Transplantation annual meeting, Keystone, Colorado, January 1995